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THE DETERMINATION OF VOLATILE FREE ACIDS IN SEWAGE BY GAS CHROM--ETC(U)
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THE DETERMINATION OF VOLATILE FREE ACIDS IN SEWAGE BY GAS CHROMATOGRAPHY USING AN INTERNAL STANDARD METHOD

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AUGUST 1981

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During the anaerobic production of methane gas from sewage, low molecular weight organic acids are also produced. Knowledge of the concentration of these acids is critical in order to maintain digester equilibrium. A method is described for the determination of volatile free acids by gas chromatography using an internal standard.

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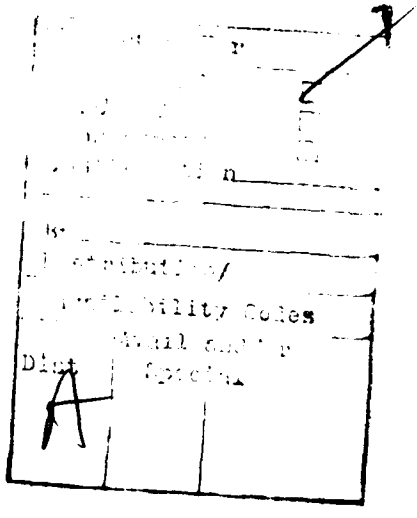

INTRODUCTION

This study was performed in partial support of an investigation of the feasibility of using anaerobic digestion to treat high pH sludges resulting from the low-level addition for phosphorus removal. The purpose of the anaerobic digestion project was to determine if existing anaerobic digesters could be used without major modifications to treat lime sludge from phosphorus removal processes in U.S. Army wastewater teatment plants. By utilizing existing processes, Army wastewater treatment plants can be improved without the expenditure of amounts of capital for process modifications.

Anaerobic digestion is a complex biochemical process in which several groups of anaerobic and facultative organisms simultaneously assimilate and break down organic matter. It is a two-phase process. In the first phase, facultative, acid-forming organisms hydrolyze and ferment the complex organic compounds to simple organic acids, the most common of which are acetic and propionic acid. The second phase involves conversion of the volatile organic acids to methane gas and carbon dioxide. The most important bacteria, the methane formers, degrade acetic and propionic acid to CO_2 and CH_4. To maintain a good anaerobic treatment system, the acid formers and the methane formers must be in a state of dynamic equilibrium. When digestion proceeds satisfactorily, volatile acids are less than 250 mg l^{-1}.[1,2] In order to monitor the volatile acids, a gas chromatographic method using an internal standard was developed.

Volatile free acids (C_2-C_5) have been determined by direct aqueous injection onto a gas-liquid chromatography column packed with Porapak N, 80/100 mesh;[3] 0.3 percent SP-1000/0.3 percent H_3PO_4 on Carbopak A;[4] 25 percent NPGS coated onto Chromosorb W;[4] Chromosorb 101 coated with 3 percent FFAP liquid phase;[5] and 15 percent SP-1220/1 percent H_3PO_4 on 100/120 mesh Chromosorb WAW.[6] Recently, Carbopak B/3 percent Carbowax 20 M/0.5 percent H_3PO_4 was developed for the separation of C_2-C_5 volatile free acids at the ppm level.[7,8]

Many investigators have reported the appearance of an "artifact peak" from distilled water, which is caused by the support materials, metal tubing, glass wool plugs, or carbonaceous deposits. It has also been reported that tailing phenomena can be minimized by using formic acid vapor in the carrier gas or by adding formic acid to the sample.[4,9-13] Van Eenaema et al. (1974) indicated that the artifact phenomenon could be reduced by adding phosphoric acid to the stationary phase or to the sample.

EXPERIMENTAL DETAILS

APPARATUS

A Hewlett-Packard 18850A gas chromatograph terminal and a Hewlett-Packard 5830A gas chromatograph equipped with a 6 ft x 2 mm ID glass column containing Carbopak C/0.3 percent Carbowax 20 M/0.1 percent H_3PO_4 and a hydrogen flame ionization detector (FID) were used for all experimental work. The operating conditions were: injection port, 200°C; column, 120°C; FID, 250°C. Helium was used for the carrier gas with a flow rate of 30 mL min^{-1}.

A Sorvall GLC-3 general laboratory centrifuge (Du Pont Instruments) was used to centrifuge the samples. A Hewlett-Packard 9810A calculator was used for the calculations. A 10-μL Hamilton syringe was used for on-column injections.

STANDARD SOLUTIONS

The following organic acids were diluted to various concentrations in glass distilled water: reagent ACS grade glacial acetic acid (Fischer Scientific Co.), purity >99.7 percent; certified propionic acid (Fischer Scientific Co.), purity 99.93 percent; isobutyric acid (J.T. Baker Chemical Co.); reagent grade butyric acid (Fischer Scientific Co.); DL-2-methylbutyric acid (Eastman Kodak Co.); and valeric acid (Eastman Kodak Co.).

PROCEDURE

Approximately 20 mL of sample were placed in a centrifuge tube and centrifuged at about 300 rpm for 30 min. Five milliliters of internal standard acid solution and 5 mL of centrifuged supernatant sample solution were mixed in a glass vial. The prepared sample was analyzed by gas chromatography to obtain the concentrations of volatile free acids using the internal standardization method.

The gas chromatographic baseline response was satisfactory after column conditioning at 175°C for 2 days with the carrier flow at 30 mL min^{-1} and injection of 5 μL of 0.1 percent formic acid in distilled water several times. Before the samples were run, the gas chromatographic baseline was checked and the linearity of response to certain standard solutions was confirmed. One microliter of sample was injected in all cases.

RESULTS AND DISCUSSION

Because the facultative bacteria for this system produced negligible amounts of butyric and isobutyric acids, butyric acid was chosen as an internal standard for the gas chromatographic determination of acetic and propionic acids. Several concentrations of each standard solution were made, and the concentration versus area was plotted to check the linearity of the standard solutions. Known concentrations of both acetic acid (or propionic acid) and butyric acid (the latter as an internal standard) were prepared and analyzed by gas chromatography, and the response factor, F, was determined. Through a knowledge of F (discussed later) and the peak area of the unknown, the concentration of the unknown was calculated from Equation (1).[14]

$$W_c = \frac{A_c \times W_a}{A_a \times F} \qquad (1)$$

where

W_a = concentration of the internal standard

W_c = concentration of the unknown acid

A_a = peak area of the internal standard

A_c = peak area of the unknown acid

F = response factor.

Depending on the content of sewage, other acids, such as isobutyric acid and methylbutyric acid, can be used as an internal standard.[14]

Each 1-μL of standard acid solution was injected at 15-min intervals, followed by one injection of 2 μL of water. The column was reconditioned by injecting 3 μL of water and 3 μL of 0.5 percent phosphoric acid in turn to obtain a satisfactory baseline response.

When silane-treated glass wool was in the injection portion, an artifact peak arising from glass-distilled water was observed between the peaks of acetic acid and isobutyric acid. In the absence of this glass wool, the artifact peak disappeared. Although an artifact peak arising from water was observed when the silane-treated glass wool was used, the peak did not overlap with other free acids.

All the data in this report represent the averages of at least five analyses under the same conditions.

All acids were easily detectable at concentrations of 1 mg l^{-1}. Figure 1 shows the gas chromatographic separation of the mixed standard acid solutions. Each standard acid solution was injected individually on the gas chromatograph to check for impurities. No impurities or interferences were found.

The standard curves of each standard solution showed correlation coefficients (r^2) of 0.999 or greater for the range of 0 to 2500 mg l^{-1} for the least squares regression of acetic, propionic, butyric, isobutyric, and methylbutyric acids; therefore, there was excellent linearity of the FID response to these acids. Only one concentration of standard solution was used to define the response factor. The standard curve for each acid solution is shown in Figure 2.

The gas chromatographic peak ratio and the concentration ratio of 365 mg l^{-1} butyric acid relative to acetic acid and propionic acid at different concentrations are presented in Figure 3. The correlation coefficients (r^2) of the curves were 0.9914 for acetic acid and 0.9990 for propionic acid, showing the excellent linearity of the FID response.

The gas chromatography peak area ratio and the concentration ratio of 544 mg l^{-1} isobutyric acid relative to acetic acid and propionic acid at different concentrations are presented in Figure 4. The correlation coefficients (r^2) of the curves were 0.9914 for acetic acid and 0.9988 for the propionic acid.

Figure 5 shows the gas chromatographic peak area ratio and the concentration ratio of 551 mg l^{-1} 2-methylbutyric acid relative to acetic acid at different concentrations. The correlation coefficients (r^2) of the curves were 0.9914 for acetic acid and 0.9990 for propionic acid at different

concentrations. The experimental data represented in Figures 3 through 5 show the validity of using either butyric, isobutyric, or 2-methylbutyric acid as an internal standard for determining acetic and propionic acids over the concentration ranges indicated.

Equation (2) shows an example of the calculation of the response factor (F) for acetic acid (AA) relative to butyric acid (BA). Values for other response factors are given in Table 1.

$$F = \frac{\text{GC area of AA}}{\text{GC area of BA}} \times \frac{\text{conc. of BA}}{\text{conc. of AA}} \qquad (2)$$

$$= \frac{37{,}003}{51{,}816} \times \frac{365 \text{ mg } l^{-1}}{563 \text{ mg } l^{-1}} = 0.463$$

TABLE 1. RESPONSE FACTORS OF ACETIC ACID AND PROPIONIC ACID RELATIVE TO DIFFERENT INTERNAL STANDARDS

Internal Standard	Factor of Acetic Acid	Factor of Propionic Acid
Butyric acid	0.463	0.794
Isobutyric acid	0.473	0.747
Methylbutyric acid	0.535	0.845

The pH of most of the sewage samples ranged between 6.0 and 7.0. Our results showed that acidification of the sample prior to injection was not necessary.

In conclusion, the results of this experiment using isobutyric acid and butyric acid as internal standards were excellent. However, when methylbutyric acid was used as an internal standard, the baseline drifted, and integration of the gas chromatograph area was not satisfactory.

It should be noted that in some cases butyric acid could be a major product of microbial degradation, depending on the actual substrate (most probably some industrial waste).

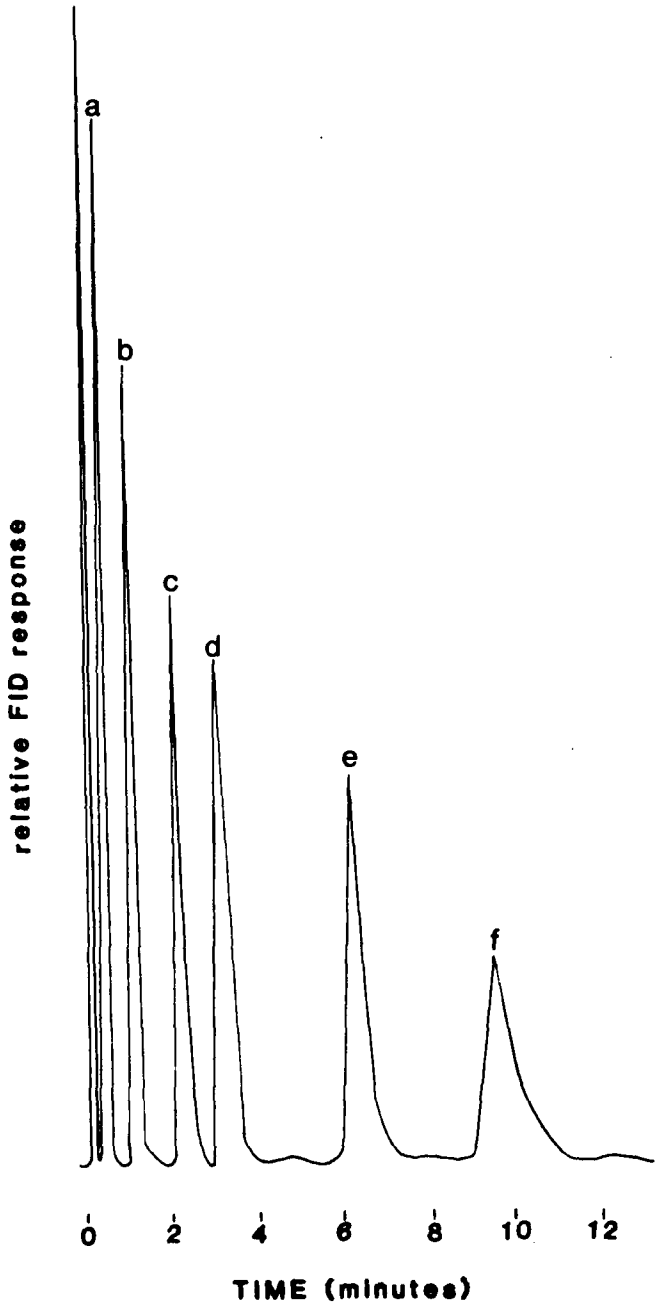

Figure 1. Separation of a mixture of volatile acids: (a) acetic acid, (b) propionic acid, (c) isobutyric acid, (d) n-butyric acid, (e) 2-methylbutyric acid, (f) n-valeric acid. Gas chromatography conditions: column, 135°C; injection port, 200°C; FID, 250°C; flow, 30 mL min^{-1}.

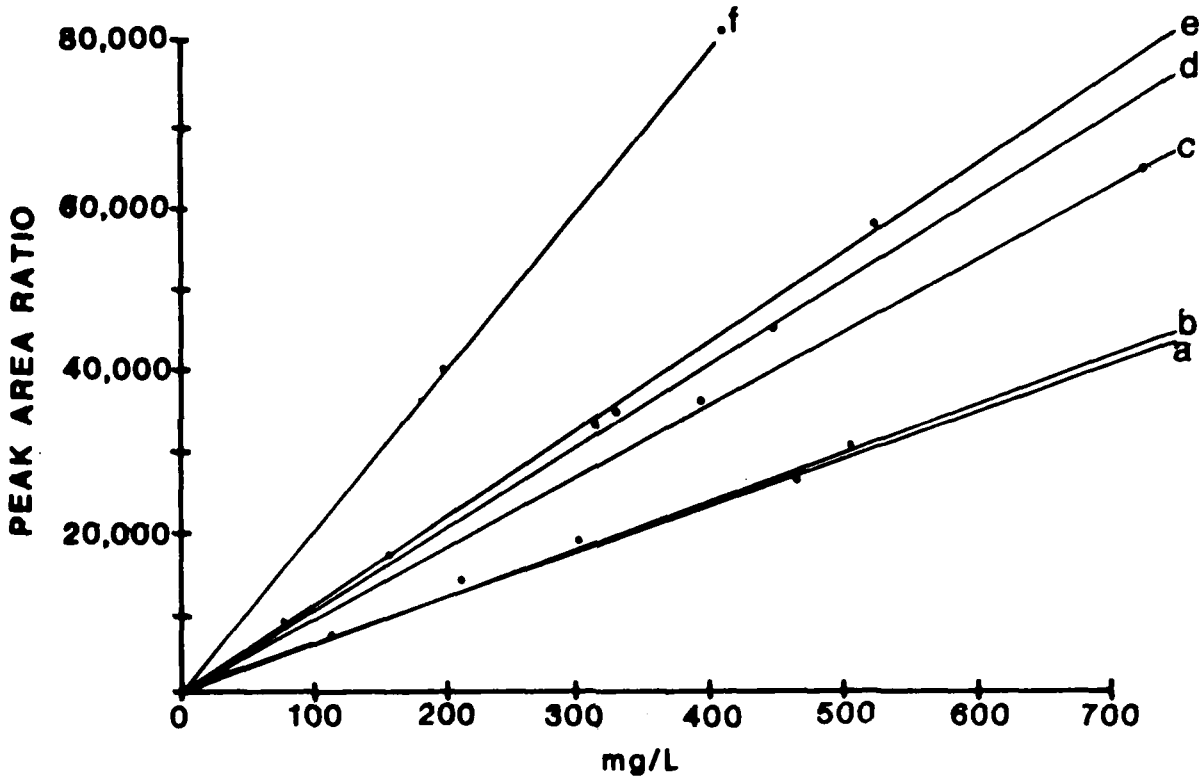

Figure 2. FID response to various concentrations of standard solutions of volatile free acids: (a) acetic acid, (b) isobutyric acid, (c) butyric acid, (d) 2-methylbutyric acid, (e) propionic acid, and (f) valeric acid.

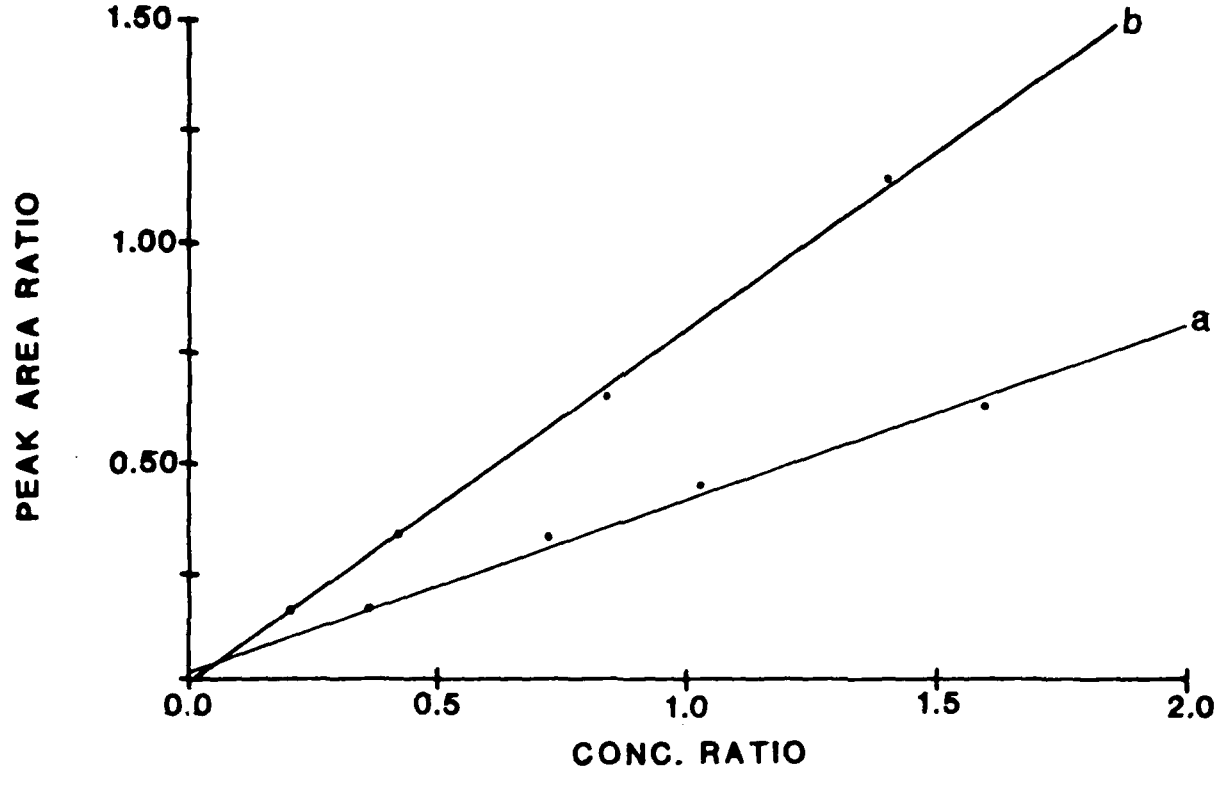

Figure 3. Linearity of response to (a) acetic acid and (b) propionic acid using butyric acid as the internal standard.

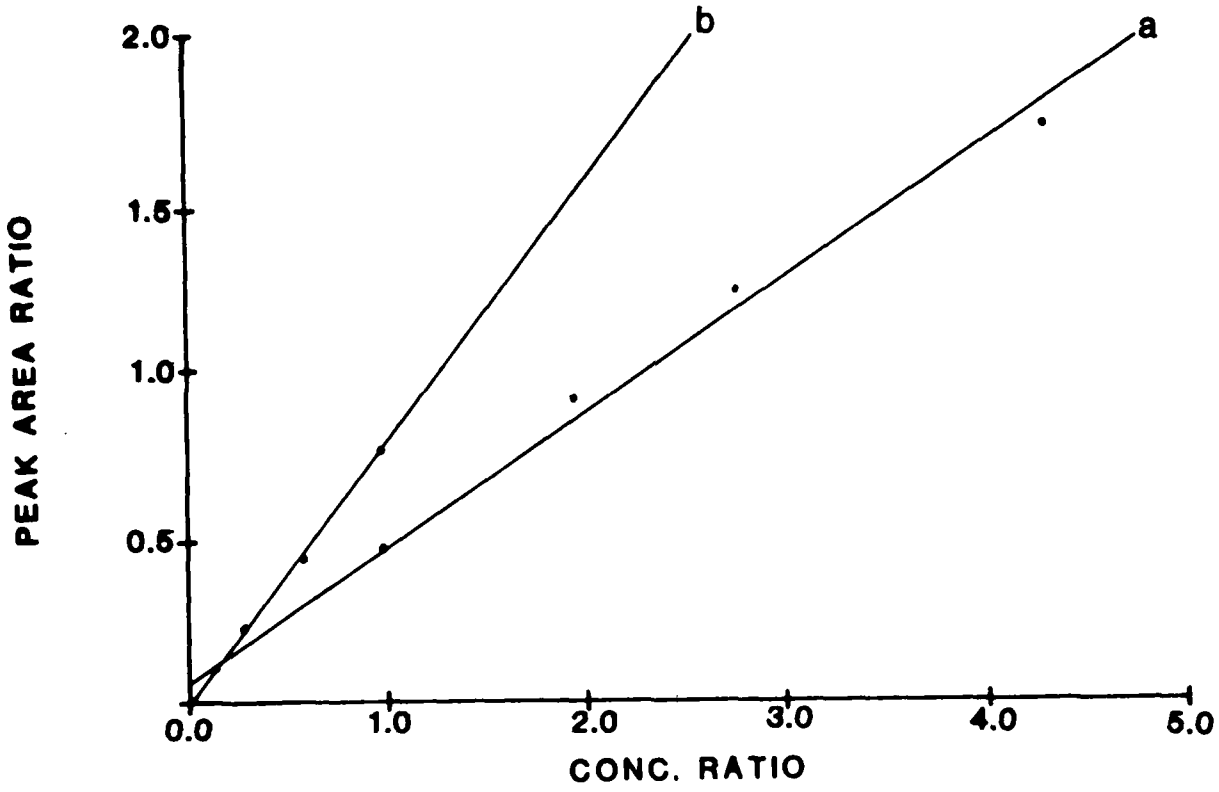

Figure 4. Linearity of response to (a) acetic acid and (b) propionic acid using isobutyric acid as the internal standard.

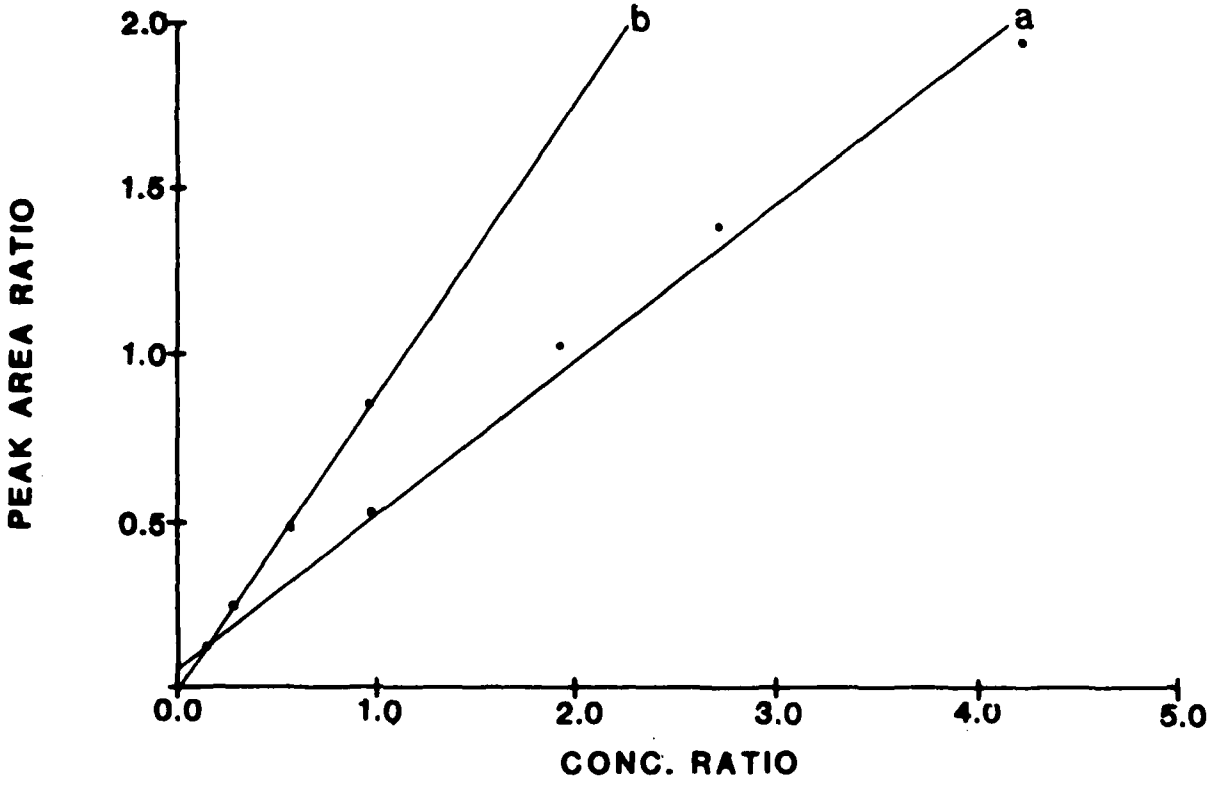

Figure 5. Linearity of response to (a) acetic acid and (b) propionic acid using 2-methylbutyric acid as the internal standard.

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